line. Remove the plate from the tank and air dry.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the red spots are from the starting line. Divide the latter by the former to calculate the $R_f$ value.

[54 FR 38375, Sept. 18, 1989; 54 FR 42886, Oct. 18, 1989]

### § 436.366 High-performance liquid chromatography assay for determining chromatographic purity of vancomycin.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers or preferably 280 nanometers;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) A 25-centimeter analytical column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles; 5 micrometers in diameter.

(b) *Reagents.*—(1) *0.2 percent triethylammonium phosphate buffer.* To 2,000 milliliters of distilled water, either add 4 milliliters of triethylamine or 4 grams of triethylammonium chloride. Adjust the pH to 3.2 with phosphoric acid.

(2) *Sample solvents.* (i) Vancomycin hydrochloride: Mobile Phase A.

(ii) Vancomycin base: 5 milliliters Mobile Phase A; add 0.1*N* HCl dropwise with swirling until sample dissolves: dilute to volume with Mobile Phase A.

(c) *Mobile Phases*—(1) *Mobile Phase A.* Add 70 milliliters of acetonitrile and 10 milliliters of tetrahydrofuran to 920 milliliters of 0.2 percent triethylammonium phosphate buffer and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase, briefly, just prior to its introduction into the chromatographic pumping system.

(2) *Mobile Phase B.* Add 290 milliliters of acetonitrile and 10 milliliters of tetrahydofuran to 700 milliliters of 0.2 percent triethylammonium phosphate buffer and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase, briefly, just prior to its introduction into the chromatographic pumping system.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of about 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the main peak (Vancomycin B) that is at least 50 percent of scale. The run time is 30 minutes per injection and the gradient conditions are as follows: (0, 12, 12.5, 8, 0, 2)

| Time (minutes) | Mobile phase A (percent) | Mobile phase B (percent) | Gradient condition |
|---|---|---|---|
| 0 | 100 | 0 | Initial conditions. |
| 12 | 100 | 0 | Isocratic region. |
| 20 | 0 | 100 | Linear ramp. |
| 22 | 0 | 100 | Isocratic region. |
| 23 | 100 | 0 | Return to initial. |
| 30 | 100 | 0 | Reequilibration. |

(e) *Preparation of resolution and sample solutions*—(1) *Resolution solution.* Prepare a solution of vancomycin hydrochloride reference standard in water containing 0.5 milligram per milliliter. Heat at 65 °C for 24 hours and allow to cool. This procedure generates two desamido-vancomycin isomers. The first desamido isomer elutes during the isocratic period and before the vancomycin B peak; the second desamido isomer elutes during the gradient ramp and is used to demonstrate the effective performance of this stage.

(2) *Sample preparation.* In a volumetric flask either dissolve a representative sample or dilute a representative portion with sample solvent to give a sample preparation containing approximately 10 milligrams per milliliter. Pipet 2 milliliters of this sample solution into a separate 50-milliliter volumetric flask and dilute to volume with sample solvent to give a diluted sample preparation containing approximately 0.4 milligram per milliliter.

(f) *Procedure.* Optimize chromatographic conditions under isocratic conditions by equilibrating the system while pumping 100 percent mobile phase A through the column. Inject 20 microliters of the resolution solution onto the column and record the chromatogram. Adjust the acetonitrile concentration of mobile phase A as needed to provide a retention time for vancomycin B of 7.5 to 10.5 minutes. Use the resolution solution to perform the system suitability tests. The elution order is resolution compound 1, vancomycin B, resolution compound 2. Return the system to the initial gradient operating conditions. Separately inject 20 milliliters of each diluted (0.4 milligram per milliliter) and concentrated (10 milligrams per milliliter) sample solution onto the column and record each chromatogram.

(g) *System suitability test.* Using the resolution solution described in paragraph (e)(1) of this section, test the performance of the chromatographic system as follows:

(1) *Asymmetry factor.* Calculate the asymmetry factor ($A$), measured at a point that is 10 percent of the vancomycin B peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

$a$=Horizontal distance from point of ascent to point of maximum peak height; and

$b$=Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor ($A_r$) is satisfactory if it is not less than 0.8 and not more than 1.8.

(2) *Efficiency of the column.* From the number of theoretical plates ($n$) calculated as described in § 436.216(c)(2) calculate the reduced plate height ($h_r$) for the vancomycin B peak as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

$L$=Length of the column in centimeters;

$n$=Number of theoretical plates; and

$d_p$=Average diameter of the particles in the column in micrometers.

The absolute efficiency ($h_r$) is satisfactory if it is not more than 40 for the vancomycin B peak in the resolution solution.

(3) *Resolution.* The resolution ($R$) between the vancomycin B peak and the peak for resolution compound 1 is not less than 3.0. Resolution compound 2 is eluted between 3 and 6 minutes after the start of the period when the percentage of mobile phase B is increasing from 0 percent to 100 percent.

(4) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$ in percent) of five replicate injections of the resolution solution is calculated as described in § 436.216(c)(4) is satisfactory if it is not more than 2.0 percent.

(5) *Capacity factor ($k$).* Calculate the capacity factor ($k$) for vancomycin B as follows:

$$k = \frac{t_r - t_m}{t_m}$$

where:

$t_r$=Retention time of solute; and

$t_m$=Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

$D$=Column diameter in centimeters;

$L$=Column length in centimeters;

$0.75$=Average total column porosity; and

$F$=Flow rate in milliliters per minute.

The capacity factor ($k$) for vancomycin B is satisfactory if it is not less than 2.6 and not more than 3.3.

When the system suitability requirements have been met, then proceed as described in paragraph (f) of this section. Alternate chromatographic conditions are acceptable provided that the system suitability parameters are met. However, the sample preparation described in paragraph (e)(2) of this section should not be changed.

(h) *Calculations.* (1) Calculate the percentage of vancomycin B in the specimen as follows:

$$\text{Percentage of vancomycin B} = \frac{A_B}{A_{Total}} \times 100 \text{ percent}$$

where:

$A_B$=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) sample solution; and

$A_{Total}$=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

(2) Calculate the percentage of each other peak as follows:

$$\text{Percentage of related substance } (i) = \frac{[A_{i/25}]}{A_{Total}} \times 100 \text{ percent}$$

where:

$A_i$=Area of any given peak, other than the main peak in the concentrated solution (10 milligrams per milliliter); and

$A_{Total}$=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

[54 FR 20383, May 11, 1989; 54 FR 25849, June 20, 1989]

### § 436.367 Thin-layer chromatographic identity test for cephalexin hydrochloride.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 × 23 × 9 centimeters, with a glass solvent trough in the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3 MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatographic plates coated with silica gel 60F–254 or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix ethylacetate, acetonitrile, water and glacial acetic acid in volumetric proportions of 42:14:18:14, respectively.

(c) *Preparation of the spotting solutions.* Prepare a solution of the sample containing 25 milligrams per milliliter of cephalexin hydrochloride in water. Prepare a solution of cephalexin monohydrate reference material at a concentration of 25 milligrams per milliliter. Add water and 0.1*N* hydrochloric acid in a dropwise mode until the material is completely dissolved.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot approximately 5 microliters of the standard solution to points 1 and 3 and approximately 5 microliters of the sample solution to point 2. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry.

(e) *Evaluation.* View the dry plate under ultraviolet light (254 nanometers). Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the $R_f$ value by dividing the latter by the former. The sample and standard should have spots of corresponding $R_f$ values of approximately 0.35.

[54 FR 48860, Nov. 28, 1989; 54 FR 51816, Dec. 18, 1989]

### § 436.368 Thin layer chromatographic identity test for cefprozil.

(a) *Equipment*—(1) *Chromatography tank.* Use a glass rectangular tank approximately 23 x 23 x 9 centimeters lined with filter paper and equipped with a tight-fitting cover.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with silica gel GF to a thickness of 250 microns.

(b) *Reagents*—(1) *Diluent.* Mix 0.1N HCl and acetone in volumetric proportions of 1:4.

(2) *Developing solvent.* Mix n-butanol, glacial acetic acid and water in volumetric proportions of 60:20:20.

(3) *Detection reagent.* Iodine vapor.

(c) *Assay solutions*—(1) *Reference standard solution.* Dissolve 50 milligrams of cefprozil (Z) reference standard in 10 milliliters of diluent.

(2) *Sample solution.* Place an amount of sample containing approximately 50 milligrams of cefprozil in a 20-milliliter glass stoppered vial. Add 10 milliliters of diluent. Shake for 5 minutes and allow the solids to settle.

(d) *Procedure.* Pour a suitable quantity of the developing solvent into a glass, chromatographic tank lined with